REMARKS

Reconsideration is requested.

Claims 1-49 have been canceled, without prejudice. Claims 50-65 have been added. Claims 51-62 are similar to now canceled claims 33-49. Claim 32 has been rewritten as claim 50, as further described below. New claims 63-65 find support, for example in Examples 2, 4, 5 and 6 of the specification.

No new matter has been added.

The Section 112, second paragraph, rejection of claim 39 is moot in view of the above. Claim 39 has been rewritten as new claim 54. Claim 54 is submitted to be definite.

The Section 102 rejection of claims 32 and 35, 39, 42 and 47-49 over Shichijo (1996, Japanese Journal of Cancer Research, Vol. 87, pages 751-756), is moot in view of the above. The claims are submitted to be patentable over the cited art and consideration of the following in this regard is requested.

The Examiner will appreciate that step (d), for example, of claim 50, recites the following:

"treating said cultured and optionally expanded cells with DNA hypomethylating agents by pulsing said cells four times every 12 hours; then replacing half of the culture medium with fresh medium and allowing to proceed for additional 48 hours so that said cells express said antigens."

Support for this recitation is found in the Examples, such as, for instance, page 20 and the same recurrence in each Example. The claimed method provides the

technical solution to obtain CTA-APC cells useful for vaccines, which was not described and/or suggested by the cited art.

The claimed invention relates to the generation of normal lymphoid cells, taken from healthy volunteers and from cancer patients, that concomitantly express *de novo* multiple Cancer Testis Antigens (CTA); the invention aims to utilize these generated cells as autologous cellular vaccines to immunize cancer patients against CTA potentially expressed on their own tumor tissues. In order to be effective in its clinical scope the invention required the development and validation of technical and methodological processes that were not taught or suggested by Shichijo et al., or any of the art of record.

Indeed, with specific relevance to the paper by Shichijo et al., the results therein obtained led the authors to experimental conclusions opposite to those drawn by the present applicants. Shichijo et al. did not provide the presently claimed method and Shichijo et al. would not have led one of ordinary skill in the art to conclude that their work could have been of any clinical, therapeutic and industrial utility for treating cancer patients affected by CTA-positive tumors. See, also the discussion of this reference in the present application at page 11 ("*...demethylation is not a sufficient stimulus to induce MAGE genes in all cases and that their results should lead to a better understanding of mechanisms of MAGE genes expression in cells. No perspective therapeutic implications were suggested*").

Shichijo et al. teach a methodology different from the presently claimed invention, and their methodology is responsible for the inconsistent data (56% positive on tested – namely 14 out of 25 - , see Table 1, line 3 in the cited reference) obtained from mRNA

levels with PBMC from healthy subjects, compared to the results obtained with the present invention (100% positive on tested - namely 28 out of 28, see Table in Example 6 in the description and in the Table filed on February 24, 2003). Moreover, the presently claimed invention has been demonstrated to obtain the same beneficial results in PBMC from cancer patients (100% positive on tested, namely 21 out of 21 – see Table in Example 6 filed on February 24, 2003).

The presently claimed methods are not described or suggested in the cited art and the results of the present invention are unexpected in view of the conclusions drawn by the authors of the cited reference based on their experimental results: "*These results suggest that demethylation is not a sufficient stimulus to induce MAGE genes in all cases*" (second column, page 755).

These striking difference is due to the presently claimed method.

In fact, as apparently appreciated by the Examiner, Shichijo et al. teach activation of PBMC from healthy volunteers by PHA and IL-2 for 4 days followed by incubation for an additional at least 8 days with PHA and IL-2 in the presence or absence of 5-aza-2'-deoxycytidine (DAC). See, pages 3-4 of the Office Action dated April 22, 2003.

The applicants have appreciated however that as DAC, for example, is an S phase drug, the applicants have discovered that it is crucial to add a hypo-methylating agent to cultures of PBMC at their peak of DNA synthesis and that repeated pulsing of cultures with, for example, DAC, and with the timing recited in the claims, to insure reproducible DNA demethylation. The claimed method provides highly consistent and reproducible (through different assays) *de novo* concomitant expression of all CTA

investigated and is not limited to members of the MAGE family but also comprise components of other families of CTA. The additional 2 days of culture in the absence of further treatment with, for example, DAC, and/or PHA+PWM have been discovered by the applicants to allow protein synthesis starting from CTA-specific *de novo* synthesized mRNA and, thus, to obtain CTA expression at protein level by cultured PBMC.

In a preferred embodiment of the presently claimed method, the activation of PBMC from healthy individuals and from cancer patients with PHA+PWM and IL-2 is carried out for 2 days (which represents the optimal timing for DNA synthesis – see Example 6) followed by an additional 2 days of culture in which cultures are pulsed 4 times (i.e., every 12 hours) with, for example, DAC, in the absence of additional treatment with PHA+PWM.

This methodology is crucial in order to obtain an efficient, concomitant and consistent (i.e., through different assays) *de novo* expression of all CTA investigated and in a short time-period on PBMC from healthy donors and cancer patients exemplified by the present disclosure.

The Examiner is urged to appreciate that the method of Shichijo et al. included a continuous DAC incubation for 8 days (page 751, right col., second paragraph). Shichijo et al. do not provide any evidence that PBMC treated under their reported experimental conditions retain immunological properties capable of stimulating autologous and allogeneic PBMC, which is a crucial parameter confirming their ability to function as vaccinating cells (See page 39, line 19 to the full paragraph, Table III).

Moreover, Shichijo et al. do not teach or describe the immunophenotypic analyses of DAC-treated PBMC. This analysis is essential to define whether the cells

treated according to the claimed method will be able to function as antigen presenting cells, which is a mandatory requirement for the cells to be useful for making vaccines.

These immunophenotypic analyses were reported in the present invention and show that DAC treatment induces quantitative modifications of cell surface molecules (e.g., HLA antigens, co-stimulatory/accessory molecules) that are crucial for their functional immunologic activity. Similar considerations apply to all the other lymphoid cells fractionated from PBMC of healthy donors utilized for the present invention. Thus, a method of mitogen activation and DAC treatment of normal lymphoid cells such as the one described by Shichijo et al. (that does not teach how to recover in a short time period lymphoid cells, retaining specific immunologic functions and immunophenotypic characteristics, and consistently and concomitantly expressing different CTA belonging to different CTA families) does not place the presently claimed invention in the hands of the public or suggest the presently claimed invention. Shichijo et al. fail to fully characterize the phenotypic and functional properties of their cells and, ultimately, to utilize these cells as therapeutic tools for the vaccination against CTA of cancer patients.

Furthermore, contrary to the present invention, Shichijo et al. do not teach the effect of their methodological approach on the *de novo* expression of CTA other than the MAGE family.

Regarding PBMC from adult T cell leukemia (ATL) patients, the Examiner in relying on the cited reference is believed to be incorrect in stating that Shichijo et al. teach collection of PBMC from "*patients with adult T cell leukemia; activating said collected cells by PHA and IL-2*". The applicant believes that Shichijo et al. teach that

these cells were incubated with DAC for 8 days and do not mention treatment with PHA and IL-2. The lack of requirement of mitogenic stimuli by these cells, before DAC treatment, is believed to clearly show that these cells are capable of self replication or at least are constitutively synthesizing DNA and, thus, cannot be in any way compared to normal T cells from PBMC. It is well-acknowledged in the art that malignant cells are much less effective than normal lymphoid cells in presenting self or non self antigens (including TAA) to the immune system and, thus, to be utilized as cellular vaccines for the treatment of cancer patients. Furthermore, ATL is a very distinctive category of T cell leukemia mainly affecting the Japanese population and with specific biologic features as compared to conventional T cell leukemia (see further below) and, thus, even more further removed from normal T cells purified from PBMC of healthy donors.

The presently claimed method treats **normal** lymphoid cells from healthy subjects and cancer patients, and not tumor cells. The fact that **normal** lymphoid cells can be obtained from cancer patients should be accepted for the simple reason that a cancer patient still has **normal** lymphoid cells.

In contrast to the Examiner's statement, Shichijo et al. do not disclose that some MAGE antigens can be expressed after DAC treatment on "*T cells, B cells, monocytes and myeloid cells from healthy and leukemia patients*". In fact, opposite to the present invention, Shichijo et al. did not utilize normal T lymphocytes **purified** from PBMC of healthy individuals.

With the exception of PBMC from ATL patients, that according to Shichijo et al. contained more than 50% T leukemic cells,[1] T cells utilized by Shichijo et al. were represented by CD3+CD4+CD8- T cell clones (TCC) established from the ocular fluid of patients with human T cell lymphotropic virus type-1 uveitis which required exogenous IL-2 and feeder cells to proliferate, and by T leukemia cell lines.

These cells are not normal T cells purified from PBMC of healthy individuals nor leukemic cells drawn from patients; rather, it is clear from their body source, viral infection, phenotypic profile, spontaneous growth in culture, and culture requirement, that these TCC and T leukemia cell lines cannot be in anyway compared to normal T cells **purified** from PBMC of healthy individuals or from cancer patients, and that their biologic features cannot be in any way compared to those of normal T cells present in PBMC from healthy volunteers and cancer patients.

A similar comment applies to the above statement from the Examiner concerning B cells, monocytes and myeloid cells. As far as B cells utilized by Shichijo et al., these were represented by B cell leukemia lines that are not normal B cells and that would not be compared with normal B lymphocytes purified from PBMC of healthy individuals or drawn from leukemia patients by one of ordinary skill in the art. Concerning monocytes and myeloid cells, these were represented in Shichijo et al. by monocyte tumor cell lines or by myeloid tumor cell lines that are not normal monocytes purified by PBMC or

[1] ATL is known to be a disease highly restricted to the Japanese population as compared to other ethnic groups and T cells from ATL are biologically significantly different from T cells of conventional T cell leukemia and thus even more removed from T cells that can be purified from PBMC of healthy subjects.

normal myeloid cells purified from peripheral blood of healthy individuals and cancer patients and are not drawn from leukemia patients.

Additionally, it is well-acknowledged in the scientific field that transformed cell lines that spontaneously grow in culture can not necessarily be useful for several experimental purposes; however, it is even clearer to the scientific community that data obtained with transformed cell lines can be extrapolated to a physiologic situation that involves non-transformed, benign cells of the same lineage. Thus, without any experimental evidence or even suggestion from the data reported by Shichijo et al., the Examiner extrapolates that DAC treatment induces some MAGE antigens on normal T cells, B cells, monocytes and myeloid cells and concludes that "*Shichijio et al. disclose the instantly claimed invention*". For the above noted reasons, the applicants respectfully submit that the Examiner's conclusions are unsupported by the cited art and/or generally advanced level of ordinary skill in the art.

Shichijo et al., for example, state that most investigated MAGE genes were not induced in the majority of T cell clones and monocyte leukemia cell lines utilized in their work (see page 753, first column, second paragraph).

As further support for the applicant's conclusions regarding the art, the applicant notes that myeloid cells are not activated by PHA and IL-2.

The above is believed to clearly demonstrate that the presently claimed invention was not taught or suggested by the cited art, either individually or in combination.

The Section 103 rejection of claim 46 over Shichijo in view of Hu (2000 Endocrinology, Vol. 141, No. 12, pp. 4428-4435) is moot in view of the above. The

pending claims are submitted to be patentable over Shichijo and Hu and consideration of the following in this regard is requested.

Hu fails to cure the deficiencies of Shichijo noted above.

Moreover, Hu does not teach that treatment with DAC and histone deacetylase inhibitors has a synergistic effect on the expression of CTA with their investigated cell types. In fact, Hu did not investigate the effect of the combined treatment on the expression of CTA on their investigated cell types nor on lymphoid cells, which are the object of the present invention.

Thus, the Examiner is again believed to have inappropriately extrapolated conclusions that are not supported by the cited references. The applicant has, for example, subsequently demonstrated that concomitant treatment with DAC and histone deacetylase inhibitors do not have a synergistic effect on the expression of other genes different from CTA. Therefore, contrary to the conclusions drawn by the Examiner that "*the invention would have been prima facie obvious to one of ordinary skill of art at the time the invention was made*", it would not have been obvious that combined treatment with DAC and histone deacetylase inhibitors would act synergistically on the *de novo* expression of CTA by lymphoid cells utilized in the present invention.

An additional apparent misunderstanding of the Examiner regarding the specific object of the present invention is also believed to be evident in the Examiner's statement that "*One of ordinary skill in the art would have been motivated to do so to increase expression of cancer testis antigens in these cells.*" The invention does not concern however the increase of the expression of CTA by lymphoid cells which are the object of the present invention. In fact, the application clearly reports how to induce their

de novo expression on investigated cells. This distinction is not one of semantics but is believed to be crucial to the invention. In fact, data are available that clearly demonstrate that treatment with DAC and/or histone deacetylase inhibitors has a different effect on the increase of the constitutive expression of different CTA expressed on different cell types. The applicant would be happy to provide this data upon the Examiner's further request for the same. The Examiner's conclusions are not believed to be supported by the art and the applicant believes that without the support of experimental data generated utilizing a specific methodology designed to investigate a specific aspect related to the modulation by DAC and/or by histone deacetylase inhibitors of the constitutive or *de novo* expression of CTA by specific cell types, such conclusions drawn by the Examiner are inappropriate.

The Section 103 rejection of claims 40, 41 and 43 over Shichijo in view of Schultze (J. Clin. Invest., Vol. 100, No. 11 (1997) pp. 2757-2765)) and McKearn (Immunological Rev. (1982), Vol. 64, pp. 5-20) is moot in view of the above. The claims are submitted to be patentable over the cited art. Consideration of the following in this regard is requested.

The cited secondary references fail to cure the deficiencies of Shichijo noted above.

The Examiner is further requested to appreciate that the presently claimed invention does not concern the activation of B lymphocytes via CD40L as described by Schultz or by PWM as described by McKearn. This comment from the Examiner is believed to further evidence an apparent misunderstanding of the specific object of the

present invention, which is not represented by the identification of different procedures or mitogens to activate lymphoid cells.

As noted by the Examiner, Schultz teaches that CD40-activated B cells are an efficient APC source. Pointing out this specific aspect by the Examiner seems at least inappropriate concerning the specificity of the present invention. In fact, the evidence that resting and activated B lymphoid cells are suitable APC is well-known in the art and was not invented by

Schultz. Schultz does not teach or suggest however that demethylation can induce *de novo* expression of CTA on CD40-activated B cells and that these so treated B cells can represent appropriate tools to vaccinate cancer patients against CTA. This specific comment, and the relative conclusions drawn by the Examiner are believed to be irrelevant as the present invention does not relate to the finding of different APC sources.

The Examiner is again requested to appreciate that a characteristic feature of the present invention relates to the particular step d) of claim 50 and pulsing with the demethylating agent.

The Examiner's conclusion that "*Shichijo et al. et al. have taught a method of generating cells presenting MAGE by demethylating activated PBMC*" is not believed to be supported by the cited art.

In fact, Shichijo et al. are believed to clearly state that the aim of their work is as follows: "*We have investigated whether DAC induces MAGE-1, -2, -3, and -6 genes and MAGE-1 protein in both normal and malignant lymphoid cells in order to better understand the mechanisms of their expression in the cells.*" (see column 1 page 751).

Furthermore, the applicant believes that Shichijo et al. do not teach that they provided a method for generating cells **presenting** MAGE by demethylating activated PBMC; rather they have clearly stated and shown that they cannot induce the expression of mRNA from investigated MAGE genes in PBMC from one healthy subject (HD1) (see legend to Figure 2) and that "*DAC induced MAGE-1, -2, -3, and -6 mRNA in PHA/IL-2- activated T cells from 4, 4, 3 and 3 of 5 healthy donors, respectively*" (see first column of page 753).

In this respect, the applicant also notes that although Shichijo et al. may mention T cells, they did not utilize purified T cells in their experimental procedures but utilized PBMC from 5 healthy donors. Additionally, Shichijo et al. clearly state that "*DAC induced MAGE-1 mRNA in PHA/IL-2-activated T cells from 4 of 5 healthy donors (not HD1), but MAGE-1 protein was difficult to detect at the protein level.*" (see second column page 753), and show representative negative data in Fig. 5 (in which MAGE-1 protein was investigated and not detected in PBMC from the healthy subject HD3 that tested positive for MAGE-1 mRNA) whose legend stresses again that "*this protein was not readily detectable in PHA/IL-2-activated T cells from all 5 healthy donors*". It will be noted that, in lane 4, PBMC from healthy subject, there is no protein; in lane 6, tumor cell line treated for 6 days, there is no protein, and in lane 7, tumor cell line treated for 8 days, there is the protein. Thus, from the experimental evidence of Shichijo, one of ordinary skill in the art would conclude Shichijo et al. did not teach or suggest a method for generating cells presenting MAGE by demethylating activated normal PBMC, as provided by in the presently claimed invention.

The person of ordinary skill in the art, following Shichijo et al.'s teaching, alone or in combination with the additionally cited art, would not have been able to recover (see Table I, page 45 of the description) in a short time period large amounts of functional cells, which retain specific immunologic functions and immunophenotypic characteristics, and that consistently and concomitantly express multiple members of different families of CTA.

Even more importantly, in light of the negative data described above, Shichijo et al. one of ordinary skill would not have concluded that these cells could be utilized as therapeutic tools to vaccinate cancer patients against CTA.

In view of the above, the claims are submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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